ETHNIC- AND GENDER-SPECIFIC EFFECTS OF SUGAR SWEETENED BEVERAGES ON SERUM URIC ACID CONCENTRATIONS AND INFLAMMATION

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ABSTRACT

Xinruo Zhang: Ethnic- and gender-specific effects of sugar sweetened beverages on serum uric acid concentrations and inflammation
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Elevated serum uric acid (hyperuricemia) increases the risk for gout, renal and cardiovascular disease. Hyperuricemia also induces inflammation in human smooth muscle and endothelial cells, reflected in the upregulation of C-reactive protein (CRP), an acute-phase reactant. Sugar-sweetened beverages (SSBs) increase SUA and CRP concentrations. However, it is not clear if SUA and/or CRP response to SSB is dependent on gender, ethnicity and genotype. Our project's main aim was to determine the ethnic-, gender- and genotype-specific effects of SSBs on SUA and CRP in Caucasian, African-American or Hispanic adults. Our study in 62 subjects showed that following SSB consumption, SUA increased by 0.66 mg/dL within 30 minutes and then gradually restored to baseline concentration. Throughout the intervention, SUA was significantly different between Caucasians and African-Americans ($p<0.05$) and men and women ($p<0.01$). The present data demonstrates that SUA response is mainly dependent on an individual's gender and ethnicity.

To my late-grandmother, Zhiqing, who was my best friend and who I love and miss dearly; and
to my parents Ketao and Shuci, and my husband Hong,
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LIST OF ABBREVIATIONS AND SYMBOLS

ABCG2	ATP-binding cassette transporter, subfamily G, member 2
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BIA	Bioelectric impedance analysis
BMI	Body mass index
CI	Confidence interval
CKD	Chronic kidney disease
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
EDTA	Ethylenediaminetetraacetic acid
FCS	Fructose Challenge Study
GLUT2	Glucose transporter 2
GLUT5	Glucose transporter 5
GWAS	Genome-wide association study
HFCS	High-fructose corn syrup
IMP	Inosine monophosphate
IRB	Institutional Review Board
MAF	Minor allele frequency
NHANES	National Healthy and Examination Survey
NRI	Nutrition Research Institution
PBF	Percent body fat

SBP	Systolic blood pressure
SLC17A1	Solute carrier, family 17, member 1
SLC2A9	Solute carrier, family 2, member 9
SNP	Single nucleotide polymorphism
SUA	Serum uric acid
UNC	University of North Carolina at Chapel Hill
WC	Waist circumference
WHtR	Waist-to-height ratio
XOR	Xanthine oxidoreductase
*	p value less than 0.05
**	p value less than 0.01

CHAPTER 1: BACKGROUND

Introduction

Sugar-sweetened beverages (SSBs) including regular soda and sport drinks are often sweetened with sucrose or high-fructose corn syrup (HFCS), both of which are major sources of dietary fructose. Frequent SSB consumers were at significantly higher risk of developing high uric acid (hyperuricemia) (1, 2), obesity and type 2 diabetes (2–4).

Fructose metabolism and uric acid

Fructose is a monosaccharide that is commonly found in fruits, HFCS. It is the only monosaccharide that raises serum uric acid (SUA) concentration (**Figure 1**). After gaining access into the jejunum by facilitative transporters glucose transporter 5 (GLUT 5) and glucose transporter 2 (GLUT 2), fructose is then transported in the portal vein to the liver to be metabolized. Fructose is phosphorylated by enzyme fructokinase-C to fructose-1-phosphate (5). Fructokinase-C, in contrast to hexokinase, is not regulated by feedback mechanism; therefore, when a large amount of fructose is consumed, adenosine triphosphate (ATP) quickly exhausts to supply the phosphate needed by fructokinase-C. The adenosine monophosphate (AMP) produced from ATP is either deaminated to inosine monophosphate (IMP) by AMP deaminase or dephosphorylated to adenosine by nucleotidase (5). As a result, the amount of uric acid produced is proportional to the amount of the fructose ingested.

Hyperuricemia is a risk factor for gout and other metabolic disorders

Uric acid is a weak organic acid that is present as monosodium urate at physiological pH. Humans do not have the enzyme uricase to oxidize uric acid to 5-hydroxyisourate and subsequently to allantoin, a water-soluble excretion product that can be eliminated by the urine. Although it has been proposed that the evolutionary loss of uricase is a selective advantage as uric acid acts as an antioxidant and neuroprotector (6), without uricase to effectively lower uric acid concentrations, humans are vulnerable to diseases such as gout because crystals form when the concentrations of SUA exceed its saturation point and deposit in tissues and joints. The concentrations of SUA are determined not only by its production but also by its elimination. One thirds of the uric acid in our system can be excreted through the intestine while the remaining two thirds are eliminated through the kidneys, (7,8). Uric acid travels in the blood to the kidney, where it is filtered through the glomerulus and then travels to the renal proximal tubule. There are many urate transporters lining on the basolateral and apical sides of the renal proximal tubule that are capable of translocating uric acid in both directions. 90% of the uric acid in the kidneys is reabsorbed back into the bloodstream by urate transporters including solute carrier camily 2 member 9 (SLC2A9), and 10% are excreted in the urine via efflux transporters including solute carrier family 17 member 1 (SLC17A1) and ATP-biding cassette subfamily G member 2 (ABCG2) (**Figure 1**).

High uric acid (hyperuricemia) is defined as a concentration of uric acid greater than 7 mg/dL in men and 5.7 mg/dL in women (9). Hyperuricemia is closely allied with metabolic syndrome (10), hypertension and cardiovascular disease (11). Furthermore, the higher the uric acid level the higher the risk of developing gout (9). In a prospective cohort study, healthy men

who had a SUA concentrations of 9 mg/dL or more had an incidence rate of gouty arthritis of 5% per year, which was almost 500-times the incidence rate of those whose SUA concentrations were below 7.0 mg/dL (12). Gout is estimated to be present in 3.9% of the population in the United States, and 5.9% in men while 2.0% in women (13).

SUA and inflammation

The double bond in uric acid makes it an anti-oxidant that is capable of scavenging free radicals. However, earlier work conducted in human vascular cells reported that physiologically relevant uric acid triggered an upregulation in C-reactive protein (CRP) mRNA and proteins (14). There has been a debate over whether there is a causal relationship between hyperuricemia and CVD ((15–17) and we speculate CRP to be the link. CRP is produced in the liver as an acute-phase response protein to nonspecific infection and inflammation (18). The median concentration of CRP in healthy adults is 0.08 mg/dL (19). Due to the advance of high-sensitivity assays, extensive studies were published demonstrating CRP as a predictive marker for future cardiovascular events (20,21). The relationship between fructose/SSB and CRP has also been explored. In an acute feeding study similar to ours, CRP increased significantly and peaked 60 minute in subjects who consumed fructose compared to those who consumed glucose or sucrose (22). Similar result was also observed in a 3-week crossover study (23).

Gender-specific effects on SUA

Women, compared to men of similar age, usually have a lower SUA concentration. Epidemiological studies have shown that both natural and surgical menopause were associated with increased SUA levels by 0.34 mg/dL and 0.36 mg/dL (24). Hormone use, on the other hand,

was associated with lower SUA levels among postmenopausal women (24–26). Furthermore, the uric acid levels in male-to-female transgender persons fell significantly after one year of hormone therapy when compared with baseline (27). Taken together, these results suggest a role of female hormones on uric acid levels that could be due to a higher renal excretion or a lower production. To this date, only a handful of studies have looked into the effects of estrogen, the primary female sex hormone, on SUA concentrations. Anton et al. reported that neither renal excretion of urate nor serum urate was influenced by estradiol-17b (28). Alternative hypotheses for such gender-specific difference in SUA levels include, menopause alters body fat distribution which leads to insulin resistance that concomitantly influences uric acid concentrations (26) and hyperinsulinemia lowers urinary uric acid excretion (29).

Ethnic-specific effects on SUA/gout and CRP

An epidemiological study of 5926 subjects showed that black persons had significantly higher SUA levels than white persons (30), which mirrors the higher prevalence of gout in blacks reported in other studies (31,32). During 1988 and 1994, analysis on NHANES III revealed that about 32% African Americans were hyperuricemic, followed by Caucasians (29%) and Mexican Americans (28.8%) (33). Outside of the United States, gout was significantly more prevalent in New Zealand Maori (6.4%) than in Europeans (2.9%) in a cohort study (34). What worth noticing is that such ethnic-specific effects overlap with many risk factors for gout including obesity and hypertension.

Interestingly, a cross-sectional study in 3154 women reported differences in CRP concentrations after multivariate adjustments including BMI (35). In this study, African-American women had a 37% increase in risk while East Asians were 50% less likely of having a

CRP concentrations higher than 0.3 mg/dL. Genetic variance of polymorphisms in CRP genes may explain such a difference.

Genotype-specific effects on SUA

60% of the variance in hyperuricemia is estimated to be attributable to heritable factors (36). A genome-wide association study (GWAS) led by Kottgen identified 28 genetic loci associated with SUA and gout in more than 1400,00 European descendants (37). Most of the genes identified encode uric acid transporters in kidneys and intestine. However, merely 7% of the variance in urate levels could be explained by GWAS (37), with the rest of variance remaining unexplained.

The effects of obesity on SUA and CRP

BMI, an indicator of body fat based on height and weight, is positively correlated with SUA (38–40) and CRP (35,41). Xanthine oxidoreductase (XOR), which catalyzes the conversion of purine to uric acid, is highly expressed in adipose tissue. Using mouse model, Tsushima et al. demonstrated that obese adipose tissue produced and secreted uric acid in a XOR-dependent manner, which was speculated to originate from obesity-related hypoxia or lipogenesis (42). Several investigators also proposed mechanisms by which adiposity impairs the clearance of uric acid that is related to insulin resistance (43,44). In these studies, hyperinsulinemia decreases urinary urate export coupled with a decrease in urinary sodium excretion, due to the fact that urate and sodium share many renal transporters (need reference). Nevertheless, an earlier study conducted in 36 obese and hyperuricemic men showed that visceral fat accumulation had little to

do with impaired urinary urate excretion, suggesting its relationship with overproduced uric acid (45).

Although a variety of studies have been conducted in each of the aspect mentioned above in relationship to uric acid, none have explored the accumulative effect of gender, ethnicity, genotype and obesity status on SUA and CRP after a fructose challenge. To fill this gap, we recruited 62 subjects from three ethnicity backgrounds in this pilot study. The methods and results are present in Chapter 2 as a journal manuscript, with the intention to publish in the future.

CHAPTER 2 JOURNAL MANUSCRIPT

Introduction

The consumption of added sugars, mostly fructose, in the United States has increased considerably in past few decades. As per the National Health and Examination Survey (NHANES) 1988 to 1994, intake of added sugars was higher in men than women and also highest in African-Americans followed by Hispanics and Caucasians (46).

Fructose has the unique role to be the only monosaccharide that raises serum uric acid (SUA) concentration. Fructose is primarily metabolized in the liver, where it is phosphorylated by fructokinase-C to fructose-1-phosphate (5). Fructokinase-C, in contrast to hexokinase, is not regulated by feedback mechanism. When a large amount of fructose is consumed, adenosine triphosphate (ATP) is quickly exhausted to supply the phosphate needed by fructokinase-C. As a result, adenosine monophosphate (AMP) gets produced and is converted into uric acid by a series of enzymatic reactions (5). SSB intake has also been associated with increased C-reactive protein (CRP), an acute phase reactant (22,23). Both elevated SUA (hyperuricemia) and CRP increase the risk for cardiovascular disease (CVD) (17,47), however, the underlying mechanism linking them to CVD risk remains less clear.

Serum concentrations of uric acid differ based on one's age, gender and ethnicity. Studies have shown that SUA is higher in non-Hispanic blacks as compared to White individuals (30). Women, compared to men in the same age, tend to have lower SUA (24). Furthermore, the

variation in SUA concentrations is strongly influenced by genetic factors. Genetic studies have identified strong association of common single nucleotide polymorphisms (SNPs) in urate transporters with altered SUA concentrations. Out of the 12 urate transporters identified so far, two urate transporters, ATP-binding cassette transporter, subfamily G, member 2 (*ABCG2*) and solute carrier family 2, member 9 (*SCL2A9*) have been studied extensively and reported to play important roles in the regulation of SUA (48).

Although a considerable amount of research has been conducted on fructose intake and subsequent uric acid production, limited is known about the ethnic- and genotype-specific effects of fructose load on SUA. The aim of the present study was to investigate the effect of a sugar-sweetened beverage (SSB) containing 80% fructose on serum concentrations of uric acid and CRP in middle-aged adults of Caucasian, African-American or Hispanic descent. We hypothesized that (1) after drinking the SSB, concentrations of SUA and CRP increase rapidly within 30 minutes and then restore to baseline levels within 3 hours, and (2) the responses of SUA and CRP to the SSB are dependent on individual's gender, ethnicity and genotype.

Methods

Subjects

The Fructose Challenge Study (FCS) is a dietary intervention study designed to investigate the acute effects of a fructose challenge on uric acid metabolism in adults of three ethnicity backgrounds. The FCS was carried out at University of North Carolina at Chapel Hill (UNC) Nutrition Research Institute (NRI) in Kannapolis, North Carolina. Men and women adults, 30 to 50 yr of age, were recruited by website advertisements and fliers posted in the Kannapolis

area. Exclusion criteria included self-reported diabetes, chronic kidney disease (CKD) and fructose intolerance. The self-reported ethnicity of the subjects was as follows: 37 Caucasians, 20 African-Americans and 5 Hispanics.

Study design

Following a 12-hour overnight fast, subjects were invited to the NRI between 0645 and 0800. All subjects were also asked to abstain from alcoholic beverages in the night prior to the study. The flow of study subjects is given in **Figure 2**.

Anthropometrics were first measured and recorded. Body composition was assessed by bioelectric impedance analysis (BIA) using Tanita Dual Frequency Total Body Composition Analyzer (DC-430U, Tokyo, Japan). Measurements were conducted in a standing position, with subjects wearing light clothing and without shoes. Height was measured to the nearest 0.1 cm by a stadiometer situated against the wall in an upright standing position. Height and weight recorded were used to calculate BMI as kg/m^2. Standard definitions were used to categorize body mass index (BMI) as underweight (BMI<18.5 kg/m^2), healthy weight (BMI 18.5-24.9 kg/m^2), overweight (BMI 25.0-29.9 kg/m^2), or obese (BMI≥30 kg/m^2). Waist circumference (WC) was measured using a stretch-resistance tape at the midpoint between the lower margin of the least rib and the top of the iliac crest to the nearest 0.1 inch. Waist-to-height ratio (WHtR) was calculated by dividing WC (inch) by height (inch). All the anthropometric measurements were taken by one staff member to minimize measurement variation and margin of error. Blood pressure was measured using an Omron digital blood pressure monitor (HEM907XL, Omron

Healthcare Inc., Lake Forest, IL). Two measurements were taken from the right arm with an interval of 1 minute and the average was used for analysis.

Next, subjects were asked to consume a SSB within 10 minutes. The drink was prepared by dissolving 60 grams of fructose (Now Foods, Bloomingdale, IL) and 15 grams of glucose (Now Foods, Bloomingdale, IL) in 300 mL of water each providing 300 kcal. Glucose was added to help alleviate symptoms of gastrointestinal discomfort caused by consuming fructose alone (49). Blood was obtained prior to the ingestion and then 30 minutes, 60 minutes, 120 minutes, and 180 minutes after the ingestion. Subjects were also asked to complete a questionnaire on demographic information, medical history, 24-hour food recall and food frequency information.

Blood processing

Blood was collected by a trained phlebotomist through venous puncture using 6-mL ethylenediaminetetraacetic acid (EDTA)-coated tubes and serum tubes (BD Vacutainer, Becton, Dickson & Company, Franklin Lakes, New Jersey). EDTA tubes were placed on wet ice, centrifuged at 3000 RPM for 15 minutes at 4°C within 2 hours of collection. Serum, plasma and buffy coat were aliquoted and stored at –80°C.

Uric acid and CRP

At every time point, the concentrations of SUA were measured using fluorometric assays according to manufacturer's instruction (Sigma-Aldrich, St. Louis, MO). CRP was measured at all five time points using ELISA (High-sensitivity, DPCR00, R&D Systems, Minneapolis, MN). Optical density was measured at wavelengths of 450 nm and 540 nm, using BioTek Synergy 2

Multi-Mode plate reader. A four-parameter logistic regression curve was generated as a standard curve to calculate the CRP concentrations of unknown samples. All samples were analyzed in duplicates.

DNA extraction and SNP genotyping

DNA was extracted from buffy coat using QIAamp DNA Blood Mini Kit (Qiagen Sciences, Valencia, CA). Concentration of genomic DNA was measured using NanoDrop Spectrophotometer. SNP rs2231142 of ABCG2 was genotyped using TaqMan SNP Drug Metabolism Genotyping Assay (Applied Biosystems, Foster City, CA). In each reaction well, 10 ng of genomic DNA was mixed with 2X TaqMan Universal PCR Master Mix and 20X Drug Metabolism Genotyping Assay Mix, according manufacturer's instruction. Real-time PCR was performed using Eppendorf RealPlex 2 Mastercycler, under the thermal cycling conditions of initial hold (95°C, 10 minutes), followed by 50 cycles of denaturation (92°C, 15 seconds) and annealing/extension (60°C, 90 seconds). SNPs rs16890979 of SLC2A9 and rs1183201 of SLC17A1 were genotyped using TaqMan predesigned SNP genotyping assay (Applied Biosystems, Foster City, CA). Each genomic DNA sample (10 ng) was amplified with 2X TaqMan Universal PCR Master Mix and corresponding 20X TaqMan SNP genotyping assay. Real-time PCR was performed using Eppendorf RealPlex 2 Masterclycler, under the thermal cycling conditions of initial hold (95°C, 10 minutes), followed by 50 cycles of denaturation (92°C, 15 seconds) and annealing/extension (60°C, 1 minute).

Statistics

All statistical analyses were performed using JMP 13.0 software (SAS Institute, Cary, NC). Results in the tables are reported as mean (standard error of the mean). After using Shapiro Wilk test to assess normality, non-normal distributed variables were either log-transformed or outliers-removed. Equal variance was assessed using Levene's test. When the equal variance assumption was violated, a Welch's t-test was conducted. The error bars in all figures represent standard error of the mean. Variables were compared between genders and obesity status using two-tailed t-test. Variables compared between more than 2 groups were analyzed using one-way ANOVA, followed by post hoc Student's t-test to account for each pair comparison. The correlations between baseline SUA and metabolic risk factors (including baseline CRP) were measured by Pearson's correlation coefficients. Statistical significance was considered as $p < 0.05$.

Ethics

This study was approved by the Institutional Review Board (IRB) of the University of North Carolina at Chapel Hill (study number 16-0876). All subjects provided written informed consent before the start of the study.

Results

Baseline characteristics

A total of 62 subjects were enrolled in the study, whose characteristics are summarized in Table 1. The subjects had a mean ± SEM age of 38.9 ± 0.87 years, BMI of 29.7 ± 0.93 kg/m^2, baseline SUA concentration of 5.5 ± 0.12 mg/dL and baseline CRP of 0.39 ± 0.07 mg/dL.

Women (n=45), compare to men (n=17), had significantly higher percent body fat ($p<0.0001$), lower systolic blood pressure ($p=0.002$) and baseline SUA ($p=0.001$). Approximately 60% of the subjects were Caucasians, 30% were African-Americans, and the remaining were Hispanics (Table 2). When stratified by both gender and ethnicity, means of body weight ($p=0.005$), BMI ($p=0.004$), percent body fat (PBF) ($p<0.0001$), waist circumference ($p=0.008$), waist-to-height ratio (WHtR) ($p=0.01$), systolic blood pressure ($p=0.001$), baseline uric acid ($p=0.005$) and CRP ($p=0.02$) differed significantly across 5 ethnic-gender groups (Table 2). Specifically, African-American women (n=15) had significantly higher adiposity-related variables (body weight, BMI, PBF, WC, and WHtR). Caucasian men, on the other hand, had the highest baseline SUA ($p<0.05$). Pearson's tests showed that SUA was positively correlated with systolic blood pressure (SBP) in only Caucasians ($r=0.43$, p0.0009; Table 3), and CRP was positively correlated with adiposity measurements, including BMI ($r=0.66$, $p<0.0001$), Percent body fat ($r=0.54$, $p<0.0001$), WC ($r=0.59$, $p<0.0001$) and WHtR ($r=0.65$, $p<0.0001$).

Serum uric acid

Serum uric acid concentration, on average, increased by 0.66 mg/dL in the first 30 minutes following SSB consumption and then gradually returned towards baseline in the remaining 2.5 hours. When adjusted for age and gender, the average concentrations of SUA varied significantly by ethnicity (Figure 3). Because of the small sample size, Hispanic were excluded from the statistical analysis in this part. Except for the baseline and end point, African-Americans had significantly lower SUA concentrations than Caucasians ($p=0.03$ at 30-min, $p=0.02$ at 60-min, $p=0.04$ at 120 min). Meanwhile, African-Americans and Caucasians exhibited significantly different percent change in SUA in the first 30 minutes post-intervention ($p=0.002$;

Figure 3B). Furthermore, men had significantly higher SUA throughout the intervention than women, even after accounting for effects of age and race (p=0.001, 0.007, 0.005, 0.005 and 0.002 at 0-, 30-, 60-, 120- and 180-min; Figure 4A). Surprisingly, instead of the first 30 minutes, the last 60 minutes was the time interval when men and women had significantly different percent change in SUA (p=0.02; Figure 4B). Precisely, SUA in men increased while SUA in women decreased between 120- and 180-min.

The effects of SNPs on SUA during the fructose challenge

We genotyped three uric acid transporter SNPs and the minor allele frequencies (MAFs) of all participants are summarized in **Table 4 I** and the MAFs stratified by ethnicity are present in **Table 4 II**. In our study, all ethnicities shared the same minor allele for all SNPs except for rs16890979, where C was the minor allele among African-Americans while T was the minor allele among Caucasians and Hispanics. After adjustment for covariates, SUA and its percent change did not differ significantly by genotype (**Figure 5**).

CRP

At the baseline, women had higher serum concentrations of CRP than men, though not significantly (p=0.16). When ethnicity was taken into consideration, African-American women had significantly higher CRP than African American men (p=0.09) and Caucasian women (p=0.01). On average, CRP stayed constant during the first 30 minutes then increased by 0.03 mg/dL and finally restored baseline levels (data not shown). CRP did not differ significantly according to gender or race. Nevertheless, obese participants (n=34) had significantly higher CRP concentrations throughout the intervention (p<0.01; Figure 6).

Discussion

This is the first dietary challenge study in Caucasians, African-Americans and Hispanics to compare serum concentrations of uric acid and CRP following an acute fructose load. Our results have demonstrated that in response to the SSB, the variation in SUA concentrations is dependent on one's ethnicity and gender.

SUA concentrations have been observed to rise after eating a large fructose-based meal (50) or drinking commercially available soft drinks (51). Indeed, in our study, SUA concentrations escalated in the first 30 minutes and then gradually declined to the baseline concentrations within 3 hours. In a study by Carran et al. (12), a soft drink as small as 355ml showed significant increase in circulating uric acid at 30min as well as 60min time points. When considering ethnicity, Caucasians had the highest SUA at baseline and throughout the intervention than participants of the other two ethnicities, which was in contrast to the results of the African-American Study of Hypertension and Kidney Disease (52). Additionally, after adjusting for age and race, there was a pronounced gender-specific difference in SUA concentrations at baseline and throughout the fructose challenge. Previous studies (24,26) have shown that the gender-specific difference decreased as women age and was eventually lost after menopause, implying a role for estrogen in uric acid excretion. In the present study, women subjects had a mean age of 39 which was younger than the average age of U.S. women experience menopause (53), possibly explaining the observed difference in SUA concentrations. In the study conducted by Dalbeth et al. (54), following a fructose load, adults with a BMI greater than 25 kg/m^2 had higher serum urate than those with a lower BMI throughout the

observation period. In the current study, such a distinct response mediated by BMI was observed but did not reach statistical significance (data not shown). Interestingly, after adjusting for the effects of age, gender and ethnicity, participants with BMIs greater than 30 kg/m^2, on average, experienced a decrease in SUA during the first 1 hour post-intervention while the non-obese counterparts had increased SUA.

Uric acid levels are modulated by numerous factors, including dietary intake of purines and fructose, endogenous production and renal and gastrointestinal excretion (55). Genetic polymorphisms in urate transporters including *ABCG2, SLC2A9* and *SLC17A1* are also postulated to be a key modulator in multiple ethnic groups. ABCG2 (56), SLC2A9 (57) and SLC17A1 (58) are urate transporters located in kidney proximal tubule cells. A missense mutation, rs2231142 Q141K, of *ABCG2* gene has been shown to be associated with higher uric acid levels in Asian (59,60), Europeans (61,62) and New Zealand Pacific Islanders (63). Another non-synonymous mutation, rs16890979-*SLC2A9* (Val253Ile) showed strong association with SUA levels in Amish (64) and American Indians (65). Finally, in Caucasians and Polynesians, *SLC17A1* polymorphism rs1183201 was strongly associated with gout (66). Given their important roles in SUA variation, these three SNPs were genotyped and the genotype-specific effects of fructose on SUA were assessed. We found that, even though there was a distinction in SUA based on one's genotype, such responses did not reach statistical significance. Our results indicated that the minor allele of rs2231142-*ABCG2* was associated with lower levels of SUA. Our result is in agreement with a study conducted in European Caucasians and Polynesians (67). Unexpectedly, carriers of the minor allele (T) of rs16890979-*SLC2A9* had the highest SUA at baseline as well as throughout the intervention, albeit not significantly. A genome-wide

association study (GWAS) in Caucasians and African-Americans showed a contrasting result that the T allele was associated with lower SUA and a lower risk for gout (61). This discrepancy could be largely due to the limited sample size of TT carriers. The SNP rs1183201-*SLC17A1* is the least studied among the 3 SNPs that we investigated in. Dalbeth et al. (68) reported a similar result that the A allele was associated with reduced serum urate concentrations following a fructose challenge in European Caucasians. However, we observed that the T carriers showed lower SUA concentrations throughout the intervention than AA carriers.

Many clinical studies, concerning fructose and systematic inflammation, were published in the past years showing conflicting results. Aeberli et al.(23) found in a 3-week intervention study that hs-CRP increased significantly after consuming 5 different SSBs. In an acute intervention, Jameel et al.(22) reported an overall increase in CRP concentrations in subjects who consumed 50 grams of fructose dissolved in 300 mL water. On the other hand, a recent 8-day randomized crossover trial demonstrated that fructose sweetened beverage did not affect CRP significantly (69). In the present study, we observed no evidence to support the hypothesis that the SSB elicits a significant increase in CRP concentration. However, CRP concentrations in serum do seem to be affected by obesity status. This was expected, as the precursor of CRP – interleukin 6 – is produced and released in part by adipose tissue.

Although a handful studies have been conducted to investigate the effect of long-term SSB consumption on inflammation, a longer observation period still merits. The main limitation of this study is our small sample size, especially in the Hispanic population. A larger sample size might be able to detect stronger genotype-specific and ethnic-specific effects of fructose on SUA

and CRP. Other limitations of this study include (1) the absence of family history on gout because uric acid has been shown to be heritable (70), (2) the SSB composition used in this study is not found in commercially available beverages, which are often sweetened with sucrose or HFCS, and (3) CRP increases with acute infection and trauma (71), thus subjects who have had upper respiratory infection or other acute illness in the past 2-3 weeks should be excluded. However, strengths of the study include a fructose challenge which overcome the errors of self-reported diet intake information and the investigation of ethnic-and genotype-specific differences in SUA response to acute fructose load.

Conclusion

Our pilot study demonstrates that SUA concentrations respond differentially based on ethnicity and gender which needs to be confirmed further in a large-scale intervention studies.

Table 1 Characteristics at baseline, all subjects[1]

	Total	Men	Women	*p*-value[2]
n	62	17	45	
Age (yrs)	38.9 (0.87)	38.7 (2.03)	39.0 (0.94)	0.72
Weight (lbs)	185.0 (5.95)	200.1 (8.83)	179.3 (7.36)	0.07
BMI (kg/m^2)	29.7 (0.93)	29.0 (1.22)	30.0 (1.21)	0.80
PBF (%)	35.0 (1.31)	25.5 (1.99)	38.5 (1.29)	**<0.0001**
WC (inch)	38.1 (0.86)	39.4 (1.27)	37.6 (1.09)	0.35
WHtR	0.58 (0.01)	0.57 (0.02)	0.58 (0.02)	0.66
SBP (mmHg)	115.0 (1.81)	123.1 (2.67)	111.5 (2.10)	**0.002**
DBP (mmHg)	73.4 (1.52)	75.4 (3.14)	72.7 (1.74)	0.45
Uric acid (mg/dL)	5.5 (0.12)	6.17 (0.28)	5.2 (0.11)	**0.001**
CRP (mg/dL)	0.39 (0.07)	0.27 (0.11)	0.44 (0.09)	0.16

1. All values present are mean (standard error of the mean).
2. Reflects comparison between men and women by two-tailed *t*-test.
3. Without any covariate adjustment.
4. Bolded p-values are less than 0.05.
5. BMI, body mass index; PBF, percent body fat; WC, waist circumference; WHtR, waist-to-height ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, c-reactive protein.

Table 2 Characteristics at baseline, 5 groups[1]

	Caucasians		African-Americans		Hispanics	
	Men	**Women**	**Men**	**Women**	**Women**	***p*-value**[2]
n	12	25	5	15	5	
Age (yrs)	37.9 (2.17)	39.4 (1.28)	40.6 (4.86)	39.6 (1.54)	35.6 (3.26)	0.76
Wt (lbs)	205.6 (8.24)	160.0 (7.34)	186.9 (23.48)	215.0 (14.01)	169.2 (15.33)	**0.005**
BMI (kg/m^2)	29.2 (1.34)	26.7 (1.28)	28.5 (2.90)	35.8 (2.15)	28.8 (2.76)	**0.004**
PBF (%)	25.6 (2.39)	35.6 (1.75)	25.2 (4.04)	43.5 (1.88)	38.6 (2.73)	**<0.0001**
WC (in)	39.5 (1.37)	34.8 (1.24)	39.0 (3.06)	42.4 (1.95)	36.5 (1.80)	**0.008**
WHtR	0.56 (0.02)	0.54 (0.02)	0.58 (0.04)	0.65 (0.03)	0.57 (0.03)	**0.01**
SBP (mmHg)	123.8 (2.62)	109.0 (2.01)	121.4 (7.13)	119.3 (4.66)	100.6 (2.20)	**0.001**
DBP (mmHg)	73.8 (3.39)	70.2 (1.28)	79.2 (7.24)	81.0 (3.55)	60.0 (4.34)	0.05
Uric acid (mg/dL)	6.43 (0.26)	5.2 (0.16)	5.6 (0.67)	5.3 (0.20)	5.2 (0.24)	**0.005**
CRP (mg/dL)	0.15 (0.04)	0.23 (0.05)	0.42 (0.21)	0.63 (0.17)	0.76 (0.29)	**0.02**

1. Means (standard errors of the mean).
2. Reflects an overall comparison between 5 groups by one-way ANOVA.
3. Without any covariate adjustment.
4. Bolded p-values are less than 0.05.
5. Wt, weight; BMI, body mass index; PBF, percent body fat; WC, waist circumference; WHtR, waist-to-height ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, c-reactive protein.

Table 3 Correlation of baseline SUA (I) and CRP (II) with metabolic risk factors

I	All (n=61)		Caucasians (n=37)		African-Americans (n=20)		Hispanics (n=4)	
Phenotype	r (CI)	p value	r (CI)	p value	r (CI)	p value	r (CI)	p value
BMI	0.13 (-0.12, 0.37)	0.31	0.24 (-0.09, 0.52)	0.15	0.11 (-0.35, 0.53)	0.63	0.85 (-0.61, 1.00)	0.15
PBF	-0.16 (-0.4, 0.10)	0.22	-0.24 (-0.52, 0.09)	0.16	0.03 (-0.42, 0.47)	0.90	0.85 (-0.60, 1.00)	0.15
WC	0.17 (-0.09, 0.40)	0.19	0.22 (-0.11, 0.51)	0.18	0.19 (-0.27, 0.59)	0.42	0.96 (-0.07, 1.00)	**0.04**
WHtR	0.1 (-0.16, 0.34)	0.44	0.13 (-0.20, 0.43)	0.45	0.17 (-0.29, 0.57)	0.47	0.99 (0.64, 1.00)	**0.01**
SBP	0.16 (-0.09, 0.40)	0.22	0.43 (0.12, 0.66)	**0.0009**	-0.18 (-0.58, 0.28)	0.44	0.98 (0.28, 1.00)	0.02
DBP	-0.06 (-0.31, 0.19)	0.65	0.17 (-0.16, 0.47)	0.30	-0.39 (-0.71, 0.06)	0.09	0.95 (-0.12, 1.00)	0.05
0 min CRP	0.12 (-0.14, 0.36)	0.35	-0.02 (-0.34, 0.31)	0.92	0.36 (-0.10, 0.69)	0.12	0.85 (-0.60, 1.00)	0.15
II	All (n=61)		Caucasians (n=37)		African-Americans (n=20)		Hispanics (n=4)	
Phenotype	r (CI)	p value	r (CI)	p value	r (CI)	p value	r (CI)	p value
BMI	0.66 (0.49, 0.78)	**<0.0001**	0.45 (0.14, 0.67)	**0.006**	0.69 (0.36, 0.87)	**0.0007**	0.98 (0.29, 1.00)	**0.02**
PBF	0.54 (0.33, 0.70)	**<0.0001**	0.49 (0.19, 0.70)	**0.002**	0.50 (0.08, 0.77)	**0.02**	1.00 (0.99, 1.00)	**<0.0001**
WC	0.59 (0.40, 0.73)	**<0.0001**	0.46 (0.16, 0.68)	**0.004**	0.63 (0.26, 0.84)	**0.003**	0.88 (-0.52, 1.00)	0.12
WHtR	0.65 (0.47, 0.77)	**<0.0001**	0.52 (0.23, 0.72)	**0.001**	0.67 (0.33, 0.86)	**0.001**	0.81 (-0.69, 1.00)	0.19
SBP	-0.12 (-0.36, 0.13)	0.35	-0.11 (-0.42, 0.23)	0.54	-0.29 (-0.65, 0.18)	0.22	0.76 (-0.74, 0.99)	0.24
DBP	0.08 (-0.17, 0.33)	0.53	0.02 (-0.31, 0.34)	0.90	-0.10 (-0.52, 0.36)	0.67	0.66 (-0.83, 0.99)	0.34

1. Data shown without any adjustment.
2. Significant p-values are bolded.
3. r, correlation coefficient; CI, confidence interval; BMI, body mass index; PBF, percent body weight; WC, waist circumference; WHtR, waist-to-height ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, c-reactive protein.

Table 4 Minor allele frequencies of three genotyped SNPs

I	SNP	Gene	AA	AB	BB	MAF
	rs2231142	*ABCG2*	GG (52)	GT (8)	TT (0)	6.67%
	rs16890979	*SLC2A9*	CC (32)	CT (23)	TT (7)	29.8%
	rs1183201	*SLC17A1*	TT (34)	AT (20)	AA (7)	27.9%
II	**SNP**	**Race**	**AA**	**AB**	**BB**	**MAF**
	rs2231142	Caucasian	31	6	0	8.1%
		African-American	19	0	0	0%
		Hispanic	2	2	0	25.0%
	rs16890979	Caucasian	25	11	1	17.6%
		African-American	4	11	5	47.5%
		Hispanic	3	1	1	30.0%
	rs1183201	Caucasian	13	17	7	41.9%
		African-American	17	3	0	7.5%
		Hispanic	4	0	0	0%

1. A: major allele; B, minor allele.
2. The numbers showing in the table present the number of participants with each genotype.
3. SNP, single nucleotide polymorphism. MAF, minor allele frequency.

Figure 1 The production and elimination of uric acid

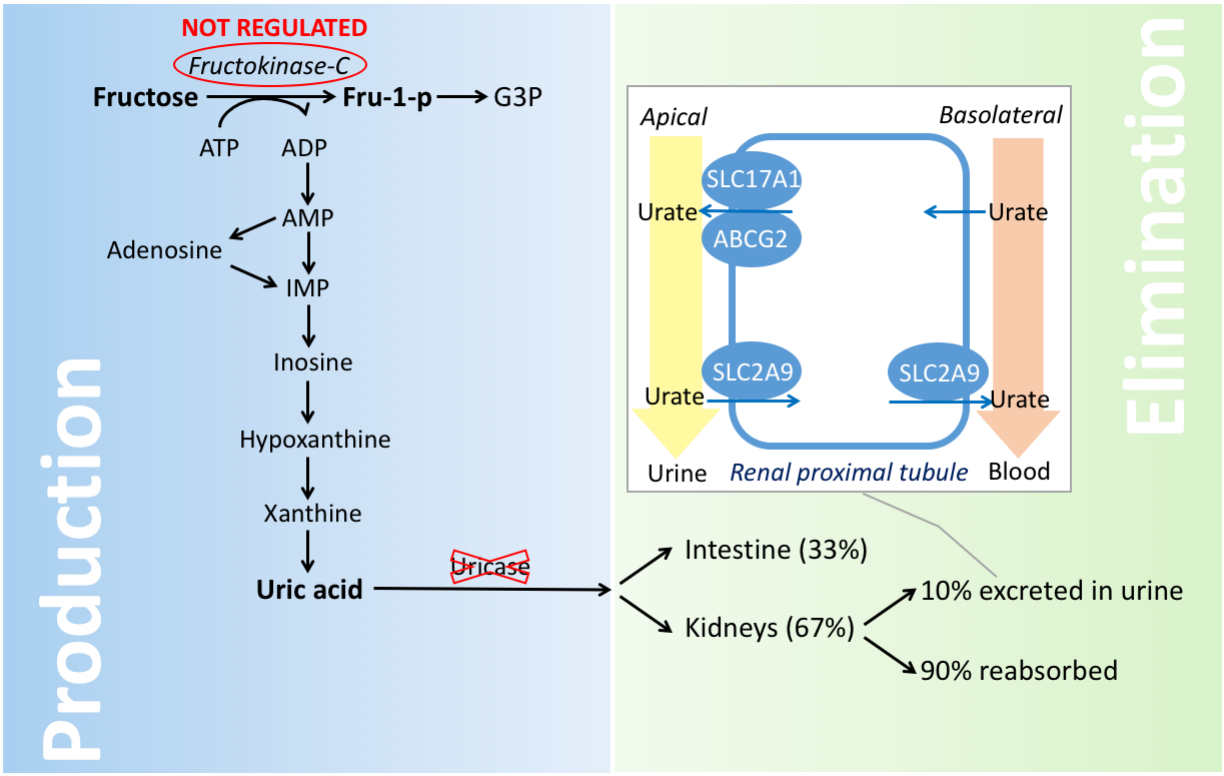


Figure 2 Flow diagram of the study

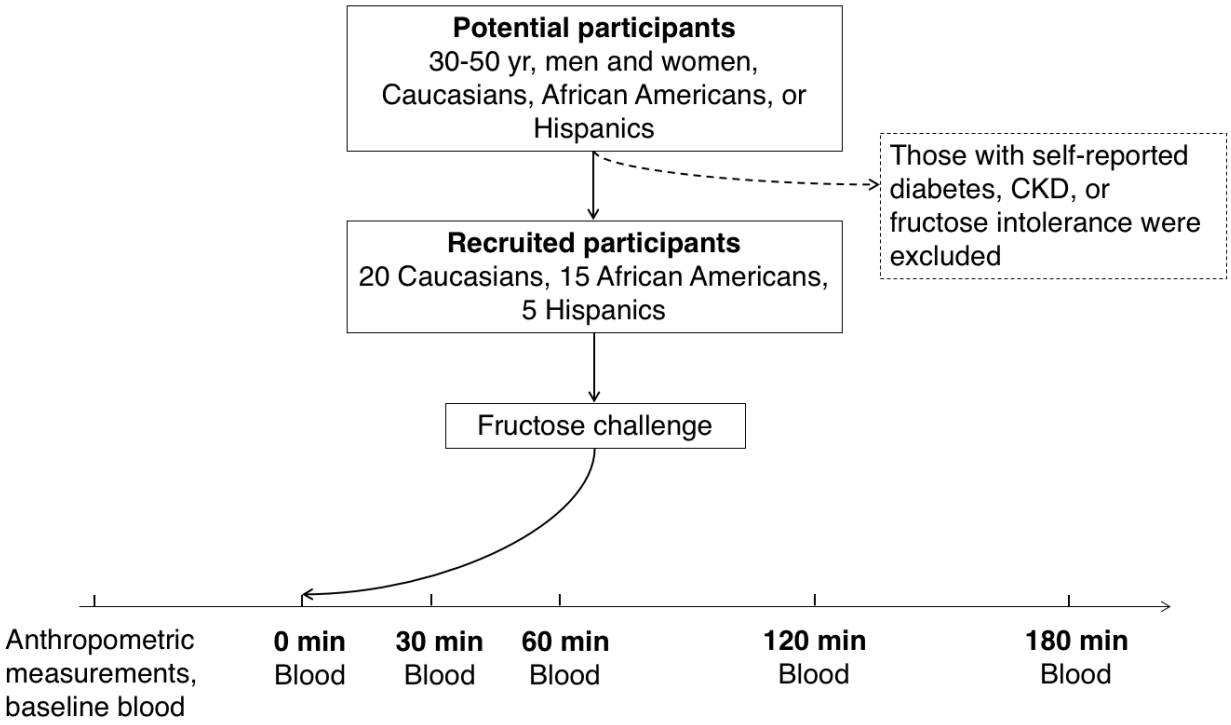
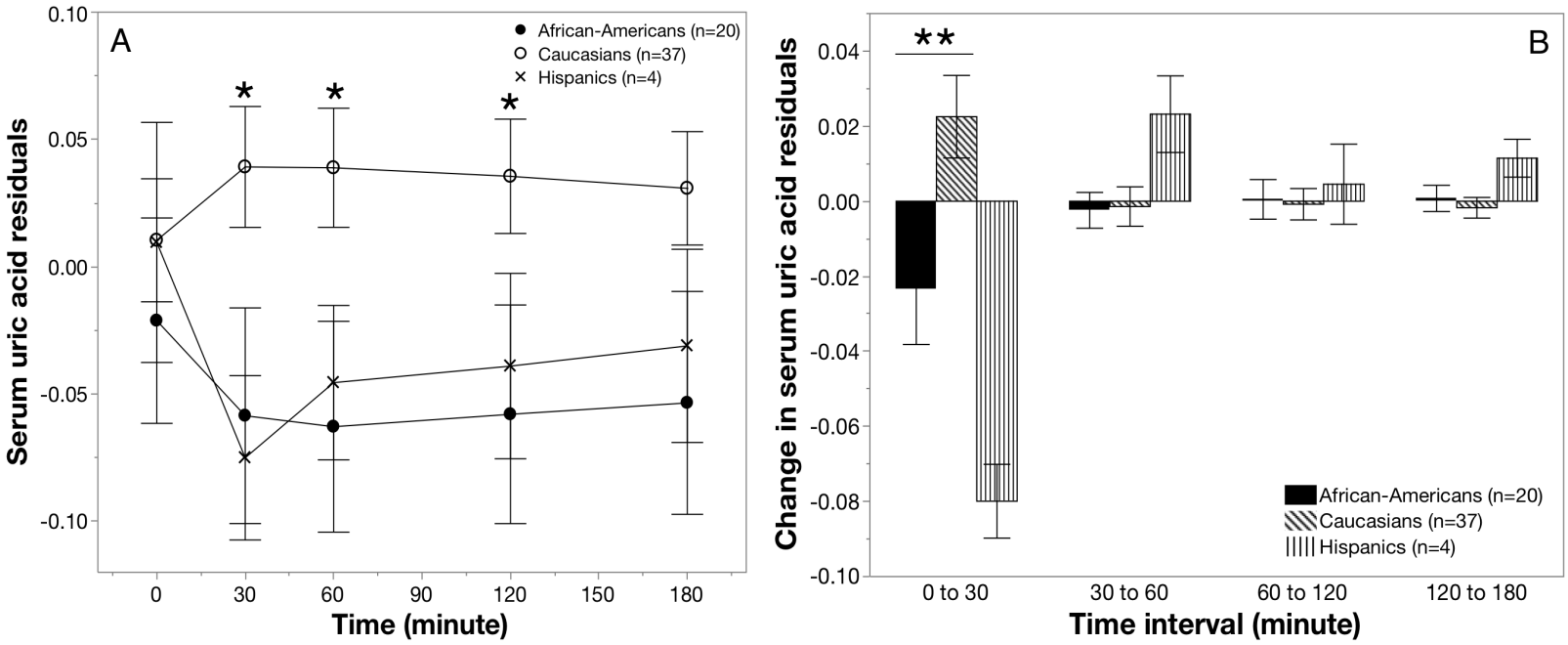
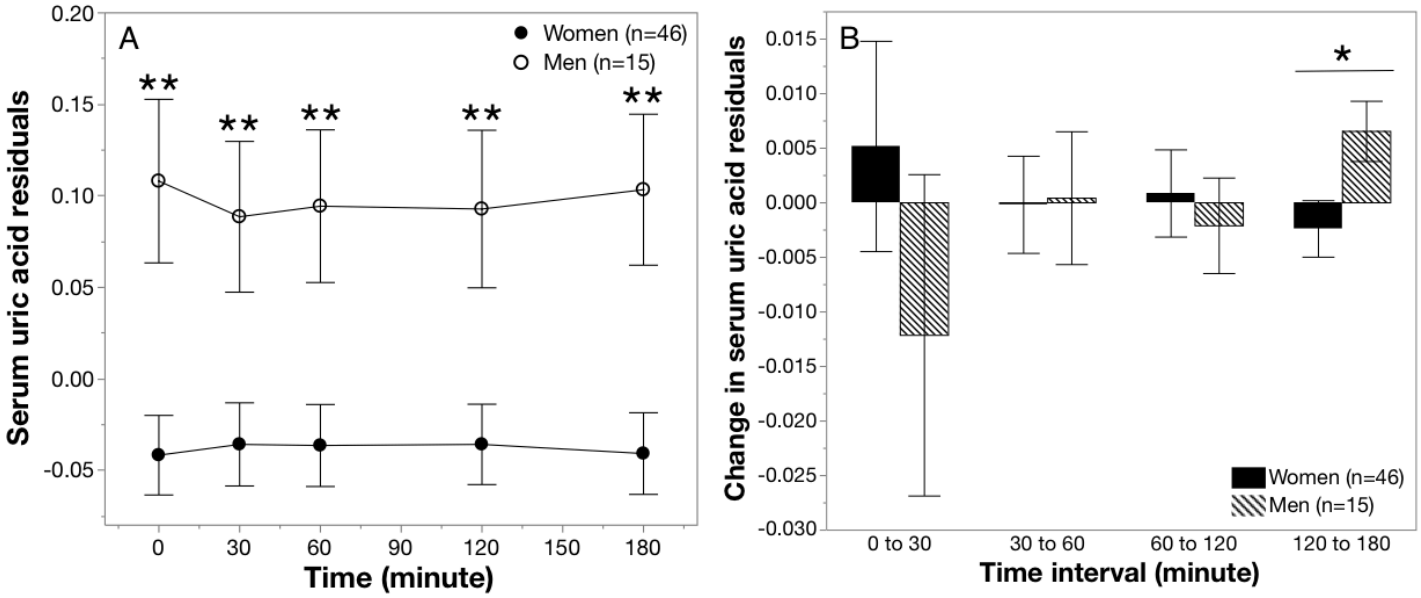


Figure 3 Serum uric acid by ethnicity



(A) Serum uric acid (mg/dL) vs. time (minute) by ethnicity, adjusted by age and gender; and (B) change in serum uric acid (%) vs. time interval (minute) by ethnicty, adjusted by age, gender and WHtR. $p<0.05$ (*) and $p<0.01$ (**) by t-test between African-Americans and Caucasians.

Figure 4 Serum uric acid by gender



(A) Serum uric acid (mg/dL) vs. time (minute) by gender, adjusted by age and ethinicty; and (B) change in uric acid (%) vs. time (minute) by gender, adjusted by age, ethnicity and WHtR. $p<0.05$ (*) and $p<0.01$ (**).

Figure 5 Serum uric acid by genotype

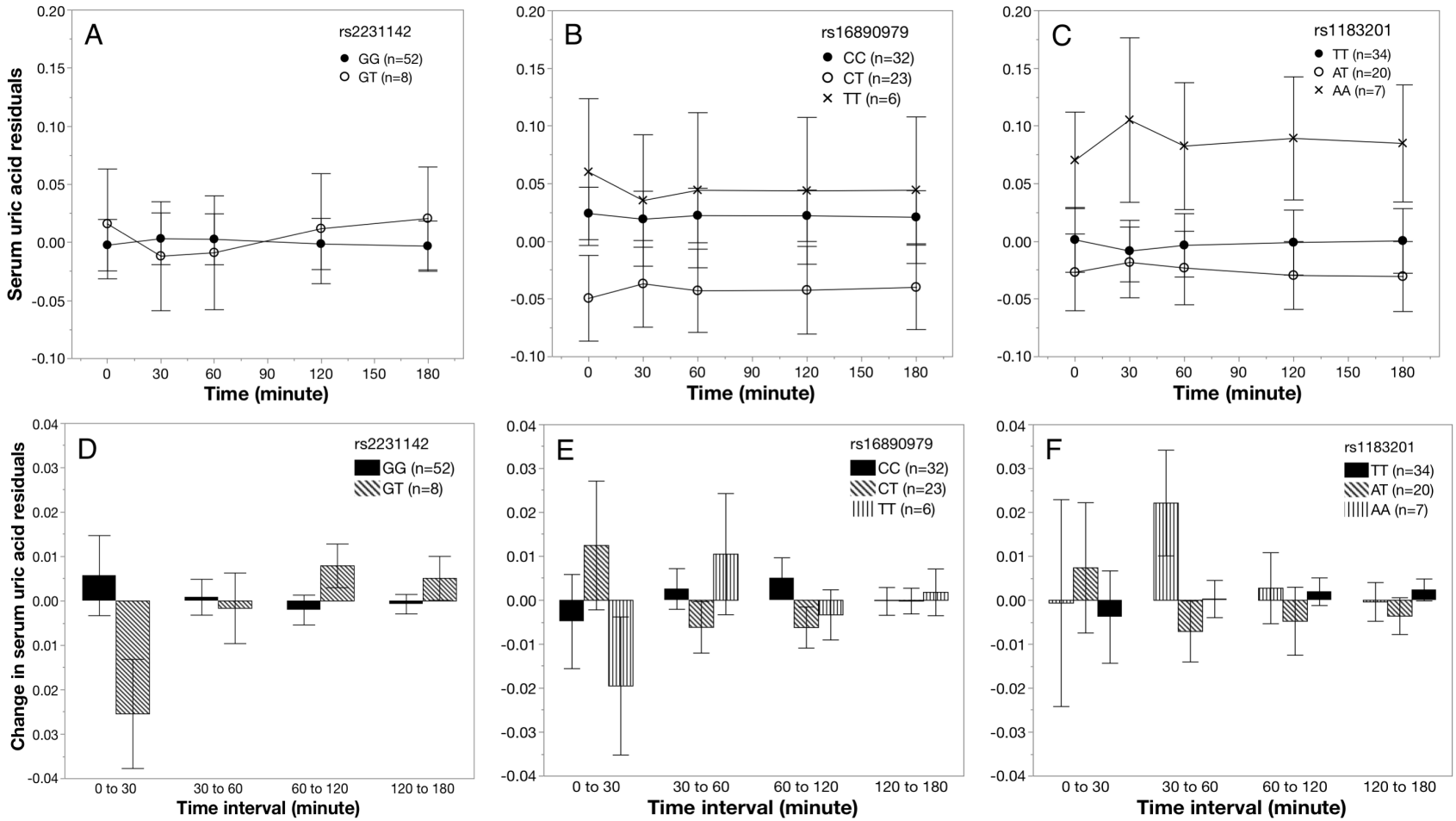
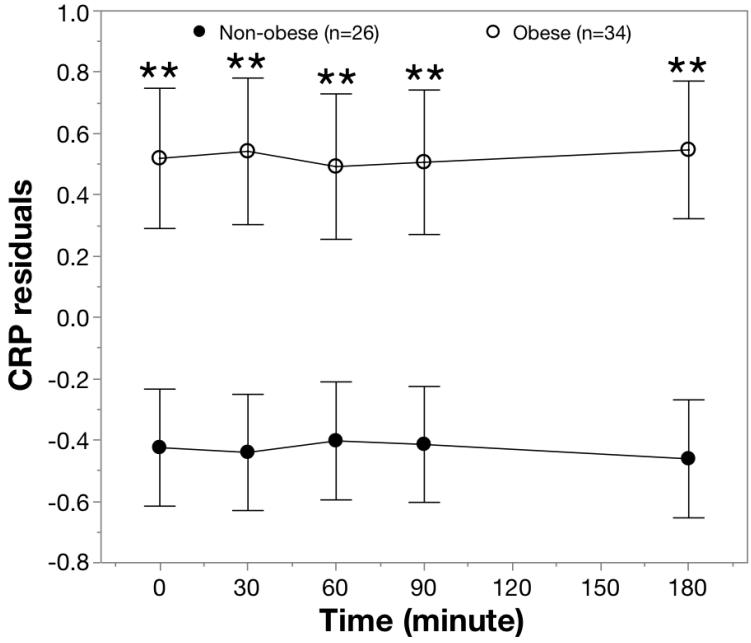


Figure 6 C-reactive protein by obesity status



CRP at each time point, adjusted by age, gender, ethnicity, smoking and alcohol. $p<0.01$ (**).

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